

Catalytic Decomposition of Cellulose under Biological Conditions

By G. HALLIWELL*

Department of Enzymology, Rowett Research Institute, Aberdeen

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1. The catalytic decomposition of undegraded cellulose in the form of cotton fibres is described with hydrogen peroxide at 0.4–0.04% (w/v) concentration in the presence of ferrous salts at pH 3–5. 2. Complete solubilization of 5 mg. of cotton fibres occurred in about 7 days in the presence of 0.4% hydrogen peroxide and 0.2 mm-ferrous sulphate at the optimum pH 4.2–4.3. 3. With 0.4% hydrogen peroxide the most rapid decomposition of cellulose was confined to ferrous sulphate concentrations of approx. 2–0.02 mm. If the concentrations of the reagents were decreased in proportion extensive breakdown occurred but much more slowly. 4. In the primary stages of breakdown cotton fibres were disintegrated to very short fibres. These were subsequently solubilized, but there was little accumulation of soluble material. Organic matter was lost from solution as the reaction progressed. 5. Other naturally occurring cellulose-containing materials, such as grass, straw, hay and sawdust, were also disintegrated and solubilized by hydrogen peroxide and ferrous sulphate.

Under most conditions cellulose consists of insoluble molecules not readily dissolved or degraded unless subjected to relatively high temperatures or extremes of acidity or alkalinity. In the laboratory a number of chemical procedures is available for this breakdown, but none can compete with the biochemical activities of cellulolytic micro-organisms. These can completely hydrolyse the most complex forms of undegraded cellulose, as found in cotton fibres, to sugars under mild conditions at about pH 4–7. In contrast, adequate chemical procedures cause extensive destruction of all material present, and produce a grossly contaminated product.

The present paper describes the complete breakdown of cotton fibres at pH 4 by very dilute hydrogen peroxide in the presence of ferrous salts in trace amounts. Disintegration of cotton to very short fibres takes place within a few days, but only small amounts of soluble products are found. The effects of a number of variables, including concentrations of ferrous salts and of hydrogen peroxide and pH, are described. The reaction on other forms of naturally occurring cellulose, namely grass, straw, hay and sawdust, was also examined.

MATERIALS AND METHODS

Substrates and reagents. Non-de-waxed and de-waxed Texas cotton fibres were as described by Halliwell (1961). Other reagents were H₂O₂ (29%, w/v; A.R.; Hopkin and Williams Ltd., Chadwell Heath, Essex) standardized against KMnO₄; m-sodium acetate–N-HCl buffer, pH 2.9–3.3; 0.2 M-sodium acetate–0.2 N-acetic acid buffer, pH 3.4–5.0. Chemicals tested for catalytic activity were: barium acetate, FeSO₄, Li₂SO₄, K₄Fe(CN)₆, K₃Fe(CN)₆ and AgNO₃ (all A.R.; Hopkin and Williams Ltd.); NH₄VO₃, BeSO₄, CdSO₄, Co(NO₃)₂, CuSO₄, CuCl, FeCl₃, MgSO₄, MnSO₄, NiSO₄, SnCl₂, SrCl₂, uranyl acetate, ZnSO₄ and zinc acetate (all A.R.; British Drug Houses Ltd., Poole, Dorset); Fe₂(SO₄)₃, mercuric acetate and SeO₂ (all Laboratory Reagents; British Drug Houses Ltd.); Cr₂(SO₄)₃ ('normal') and Al(NO₃)₃ (British Drug Houses Ltd.). All were tested at the same and double the molar concentration of ferrous salt in the standard procedure (see below).

Standard procedure for the decomposition of cotton fibres by hydrogen peroxide and ferrous salt. A 4 ml. portion of 0.2 M-sodium acetate–0.2 N-acetic acid buffer, pH 4.2, and 5 mg. of cotton fibres were mixed with water, 0.2 ml. of 10 mM-FeSO₄ and 4 ml. of 1% H₂O₂ (final vol. 9 ml.) and incubated at 37° for the desired period. The vessels were not shaken but the contents were mixed twice daily. Residual cellulose was estimated colorimetrically on filter-sticks (porosity 2) after removing soluble material and washing the residual cellulose with water (Halliwell, 1958, 1962). The washed fibres were first heated at 99° for 3 min. in 2 ml. of 0.8 N-H₂SO₄ to remove a reddish deposit

* Present address: Biochemistry Section, Department of Applied Microbiology, University of Strathclyde, Glasgow.

sometimes present after incubation with iron salts. The treated fibres were subsequently separated from the acid digest, washed with water and oxidized with dichromate. The preliminary acid treatment produced no significant change in weight of non-de-waxed or of de-waxed cotton fibres, either before or after incubation of these fibres in the peroxide-ferrous sulphate system described below (Fig. 3).

Determination of hydrogen peroxide. This was done with the CdI_2 -starch reagent described for dichromate (Halliwell, 1960). The H_2O_2 -containing solution (up to 4 ml.) was mixed with 4 ml. of 6.25N- H_2SO_4 and the reaction started by adding 2 ml. of the CdI_2 -starch reagent. After 20 min. at 25° the colour was read against water in a Spekker absorptiometer (Hilger) in a 1 cm. light-path with Ilford orange filters (no. 607, peak transmission $600\text{ m}\mu$). The calibration curve is linear in the range 0.6 – $6\text{ }\mu\text{g.}$ of H_2O_2 . For a series of tubes the CdI_2 -starch reagent was added at suitably spaced intervals of about 1 min.

Determination of total carbon. In some experiments carbon determinations were made on the insoluble and soluble material present after incubation by using the procedure and apparatus of Heald (1951). For this purpose the final concentration of acetate buffer in the reaction mixture was decreased from its standard value of about 0.1 M to about 0.2 mM .

Determination of volatile acids. These were steam-distilled in the Markham (1942) apparatus and the distillate was titrated with dilute alkali from a Conway micro-burette. A 2 ml. sample was washed into the Markham apparatus with 1 ml. of saturated MgSO_4 , 1 ml. of conc. H_2SO_4 and 1 ml. of water, and steam-distilled. Complete recovery of 3 mg. of formic acid or of 3 mg. of acetic acid was obtained in 75 ml. or in 50 ml. of distillate respectively.

Determination of carbonyl compounds. This was based on the procedure of Friedemann & Haugen (1943).

RESULTS

Fig. 1 shows that dilute hydrogen peroxide is relatively stable in the absence of added ferrous salt between pH 3.2 and 5.2. Up to pH 3.6 and in the presence of ferrous sulphate at a final concn. 0.22 mM the decomposition of hydrogen peroxide proceeded more vigorously, giving 95% breakdown in 4 days. The stability of hydrogen peroxide in solutions containing ferrous sulphate was greater at pH 4.2–5.2 than at pH 3.6, but less than in the absence of ferrous sulphate.

The conditions described in Fig. 1, ranging from slow decomposition of hydrogen peroxide at pH 3.2–5.2 in the absence of added ferrous sulphate to relatively rapid decomposition at low pH values in the presence of ferrous sulphate, were applied to the destruction of non-de-waxed cotton fibres (Fig. 2).

Peak solubilization of the cotton fibres occurred at about pH 4.2–4.3 in 2 and 4 days at 37° , when almost 90% of the original cellulose was dissolved in the longer period. The limiting factors appear to be reaction of hydrogen peroxide with cellulose and decomposition of residual hydrogen peroxide. Filtrates from the filter-sticks were free from

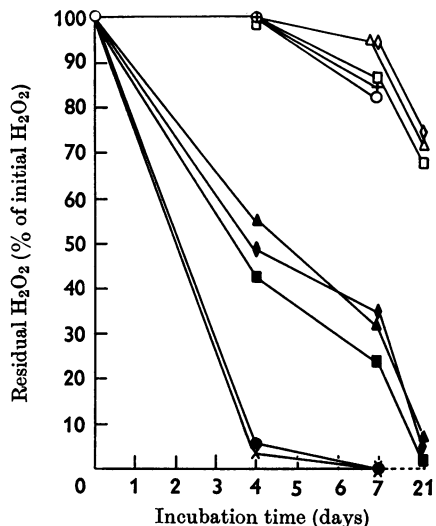


Fig. 1. Decomposition of H_2O_2 in the presence and absence of FeSO_4 at different pH values. A 4 ml. portion of 0.2 M -acetate buffer, 4 ml. of 1% H_2O_2 , 0.2 ml. of 10 mM-FeSO_4 and water to 9 ml. were incubated at the pH values shown at 37° . Residual H_2O_2 was measured colorimetrically: pH 3.2 with (●) and without (○) FeSO_4 ; pH 3.6 with (X) and without (□) FeSO_4 ; pH 4.2 with (▲) and without (△) FeSO_4 ; pH 5.2 with (◆) and without (◇) FeSO_4 .

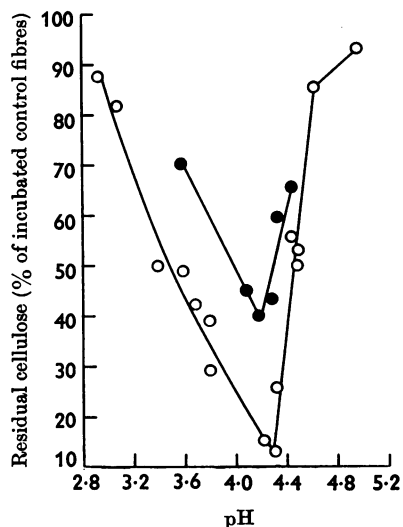


Fig. 2. Effect of pH on the solubilization of cotton fibres by H_2O_2 and FeSO_4 . A 5 mg. portion of non-de-waxed cotton fibres, 4 ml. of 0.2 M -acetate buffer, pH 2.95–5.0, 0.2 ml. of 10 mM-FeSO_4 and 4 ml. of 1% H_2O_2 (final vol. 9 ml.) were incubated for 2 (●) and 4 (○) days at 37° . Residual cellulose was determined colorimetrically on filter-sticks as a percentage of incubated control fibres.

suspended particles and opalescence. Below pH 3 and above pH 5 less than 12% of the substrate was solubilized and no loss of weight took place in 6 days when either hydrogen peroxide or ferrous sulphate was omitted from the reaction mixtures at pH 3.1–5.0.

At the pH for maximum rate of breakdown, about 4.2, little visible change took place in the first day, but during the next 24 hr. numerous short fibres appeared, which were retained by the filter. By the end of the third day the bulk of the original cotton fibres was almost completely disintegrated into short fibres, leaving the residual cotton as a very weak and extended mass of fibres that disintegrated on vigorous shaking. Subsequent degradation of these residual cotton fibres was rapid and accompanied by dissolution of the short fibres.

De-waxed cotton fibres were also converted into short fibres and soluble products, although not as readily as were non-de-waxed fibres. Thus in the experiment shown in Fig. 3 92% solubilization of non-de-waxed fibres was achieved in 7 days, compared with 75% for de-waxed fibres. The rate

of breakdown of each substrate was somewhat similar to the corresponding rate of decomposition of hydrogen peroxide.

Degradation of cellulose proceeded more rapidly at 37° than at lower temperatures, but slow breakdown also took place at 3° in reaction mixtures transferred from temperatures of 37° and 20° as well as in mixtures held initially at 3° (Fig. 4).

Effect of nature and concentration of reagents on the decomposition of cotton fibres. A final concentration of approx. 0.03M-sodium acetate–0.06N-acetic acid buffer was used at the optimum pH 4.2 for most experiments, although acetate concentrations down to 2mM have proved equally effective provided that pH was maintained. Other buffer solutions were used, at about pH 3.8–4.2, namely 50mM-potassium hydrogen phthalate alone or adjusted with sodium hydroxide, 0.2M-disodium hydrogen phosphate–0.1M-citric acid and 0.2M-disodium hydrogen phosphate–0.1M-phosphoric acid, but none gave more than 7% solubilization in 8 days.

Larger amounts of hydrogen peroxide than that used in the standard procedure proved beneficial in accelerating the degradation of cotton fibres. With three times the concentration of hydrogen peroxide in the standard procedure the visible rate of reaction approximately doubled, resulting in the production of short fibres in 24 hr. By the second

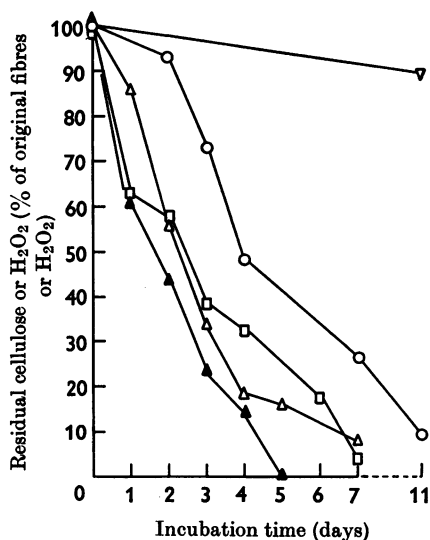


Fig. 3. Degradation of non-de-waxed and de-waxed cotton fibres by H_2O_2 and FeSO_4 . The standard procedure was carried out in acetate buffer, pH 4.2, with 5mg. of cotton fibres. Residual cellulose was determined colorimetrically on filter-sticks. Residual cellulose of non-de-waxed cotton fibres (Δ) and its residual H_2O_2 (\blacktriangle), and residual cellulose of de-waxed cotton fibres (\circ) and its residual H_2O_2 (\square), are shown. Reaction mixtures containing non-de-waxed or de-waxed fibres incubated for 11 days without FeSO_4 or H_2O_2 showed no loss of fibres. Residual H_2O_2 in reaction mixtures containing de-waxed or non-de-waxed fibres without added FeSO_4 (∇) is also shown.

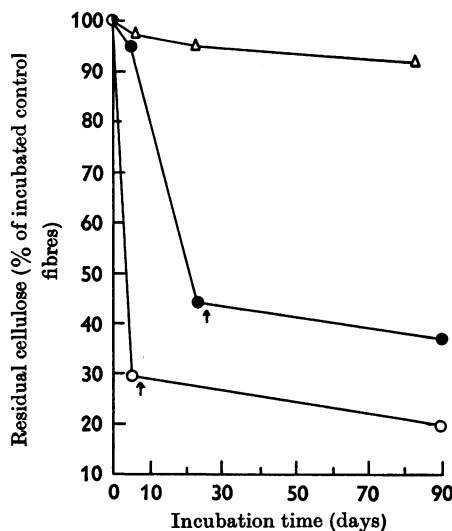


Fig. 4. Effect of temperature on the decomposition of cotton fibres in the presence of H_2O_2 and FeSO_4 . The standard procedure was carried out in acetate buffer, pH 4.2, with 5mg. of non-de-waxed cotton fibres. Residual cellulose was determined colorimetrically on filter-sticks. Reaction mixtures were at 3° (Δ), at 20° until transferred to 3° at \uparrow (\bullet), and at 37° until transferred to 3° at \uparrow (\circ).

Table 1. *Effect of hydrogen peroxide concentration on the breakdown of cotton fibres to soluble products*

The standard procedure was carried out with 5mg. of non-de-waxed cotton fibres at pH4.2 and 3.8 with the H_2O_2 concentrations shown. Residual cellulose was measured colorimetrically by filtration and oxidation on filter-sticks.

| Final concn. of H_2O_2 (%) | Residual cellulose (% of incubated control) | | | |
|------------------------------------|---|----------|--------|---------|
| | pH4.2 3 days | pH3.8 | | |
| | | 3-3 days | 4 days | 14 days |
| 0.4 | 29 | 44 | — | — |
| 0.2 | 75 | 54 | — | — |
| 0.1 | 90 | — | 64 | — |
| 0.05 | 94 | — | — | 99 |

day complete conversion into short fibres had occurred, with some of these fibres already undergoing solubilization.

The minimum concentration of hydrogen peroxide required for approx. 90% solubilization, measured by weight loss of non-de-waxed cotton fibres in 4 days, was that (0.4%) used in the standard procedure (Fig. 2). In parallel experiments at pH3.8 and 4.2 concentrations of hydrogen peroxide below 0.4% decreased the rate of breakdown of cotton fibres under both sets of conditions (Table 1). At 0.2% hydrogen peroxide concentration, in contrast with 0.4% hydrogen peroxide, solubilization of cotton cellulose was apparently more effective at pH3.8 than at pH4.2, perhaps owing to enhanced stability of ferrous sulphate at the lower pH.

At pH 4.2 with concentrations of hydrogen peroxide as low as 0.05%, slight solubilization of fibres still occurred to an extent of 6% in 3 days. Repeated additions, however, of 0.05% hydrogen peroxide (final concentration each time) at suitable intervals enabled almost 50% of the non-de-waxed cotton fibres to be solubilized in the 28-day period. The total amount of hydrogen peroxide added in stages to effect this degree of solubilization corresponded to half that added in the standard procedure where the fibres suffered 65% breakdown in 4 days (○ in Fig. 5).

The range of significant and rapid breakdown of cotton cellulose by 0.4% hydrogen peroxide is limited by ferrous sulphate concentrations of approx. 2.2–0.022mm (Table 2).

Under optimum conditions at pH4.2 in the standard procedure with 0.22mm-ferrous sulphate, 77% of the cotton fibres was dissolved within 3 days, whereas smaller and larger concentrations of ferrous sulphate slowed the breakdown process. With ferrous sulphate at three- to ten-fold the

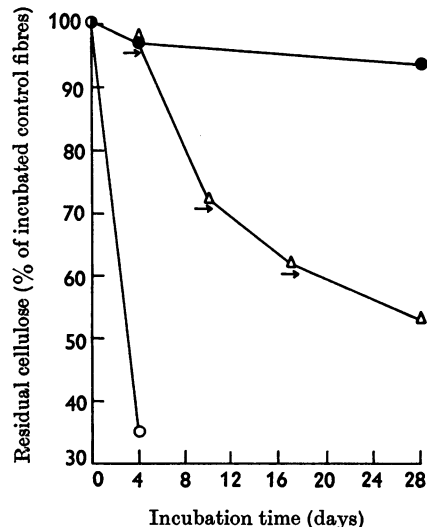


Fig. 5. Action of single and repeated additions of small concentrations of H_2O_2 on the decomposition of cotton fibres. The standard procedure was carried out in acetate buffer, pH4.2, with 5mg. of non-de-waxed cotton fibres and: ○, 4ml. of 1% H_2O_2 and no further additions, final vol. 9ml.; ●, 0.5ml. of 1% H_2O_2 and no further additions, final vol. 9ml.; △, 0.5ml. of 1% H_2O_2 and three subsequent additions of the same amount (0.1ml. of 5% H_2O_2) at →, final vol. 9.0–9.3ml.

Table 2. *Effect of ferrous sulphate concentration on the breakdown of cotton fibres to soluble products by hydrogen peroxide*

The standard procedure was carried out with 5mg. of non-de-waxed cotton fibres and 0.22mm- $FeSO_4$ at pH4.2. Variations on the standard procedure show the effect of $FeSO_4$ concentration and of pH.

| Final concn. of $FeSO_4$ (mm) | Residual cellulose (% of incubated control after 3 days) | |
|-------------------------------------|--|-------|
| | pH4.2 | pH3.8 |
| 2.2 | 82 | — |
| 1.1 | 62 | 68 |
| 0.66 | 56 | 57 |
| 0.22 | 23 | 47 |
| 0.055 | 50 | 65 |
| 0.022 | 81 | 86 |
| 0 | 100 | 100 |

concentration in the standard procedure the mixture gave reddish-brown precipitates within 2hr. of preparation, and this presumably accounted for the smaller degree of breakdown of the substrate. There is probably loss of both hydrogen peroxide and catalyst.

The conditions presented in the standard procedure were designed to achieve dissolution of a major portion of cotton cellulose in a few days, but it was also possible to produce a more leisurely and controlled breakdown of this substrate by smaller concentrations of both hydrogen peroxide and ferrous sulphate (Table 3). Under these conditions with a quarter of the standard amount of hydrogen peroxide and one-quarter to one-tenth of

the standard amount of ferrous sulphate it was possible to obtain 35–47% solubilization of fibrous cellulose within 25 days. Still smaller amounts of hydrogen peroxide, at 0.04% concentration, were also active in presence of one-quarter to one-tenth of the standard amount of ferrous sulphate in producing about 15% dissolution of cotton cellulose within 25 days. In contrast, almost the same amount of hydrogen peroxide (0.05%) but with the standard concentration of ferrous sulphate (Fig. 5) produced only 6% solubilization in the same period. Thus the ferrous sulphate concentration exerts a regulating action on the relative decomposition of hydrogen peroxide and cellulose.

A number of other chemicals were tested as substitutes for ferrous salts, namely cuprous chloride, copper sulphate, ferric chloride, ferric sulphate, manganese sulphate, magnesium sulphate, lithium sulphate, zinc sulphate, zinc acetate, cobalt nitrate, silver nitrate, barium acetate, mercuric acetate, cadmium sulphate, chromic sulphate, aluminium nitrate, selenium dioxide, potassium ferricyanide, potassium ferrocyanide, strontium chloride, stannous chloride, beryllium sulphate, nickel sulphate, ammonium vanadate and uranyl acetate. Of these only ferric salts gave results remotely comparable with those obtained

Table 3. Breakdown of cotton fibres with various concentrations of hydrogen peroxide and ferrous sulphate

The standard procedure was carried out with 5mg. of non-de-waxed cotton fibres at pH 4.2 for 3 days and also with smaller amounts of H_2O_2 and $FeSO_4$ for 25 days.

| Final concn. of H_2O_2 (%) | Final concn. of $FeSO_4$ (mm) | Residual cellulose (% of incubated control) | |
|------------------------------------|-------------------------------------|--|---------|
| | | 3 days | 25 days |
| 0.4 | 0.22 | 34 | — |
| 0.1 | 0.055 | — | 65 |
| | 0.022 | — | 53 |
| 0.04 | 0.055 | — | 82 |
| | 0.022 | — | 84 |

Table 4. Solubilization of naturally occurring forms of cellulose by hydrogen peroxide and ferrous sulphate

Approx. 25 mg. of non-de-waxed cotton fibres and 100 mg. of each other substrate (air-dried weights) in 10 ml. of acetate buffer, pH 4.2, were treated with (a) $FeSO_4$ at 0.22 mm final concn., or (b) H_2O_2 at 0.4% final concn. or (c) $FeSO_4$ at 0.22 mm final concn. + H_2O_2 at 0.4% final concn.; the final volumes were 18 ml. Samples were incubated at 37° for 21 days without further additions, or after 7 days were treated a second time with the same amount of H_2O_2 as that given initially (final vol. 18.8 ml.) and incubated for a further 14 days. Straw and sawdust were also given treatment (c) but with $FeSO_4$ at 0.44 mm and 0.11 mm final concn. After incubation the residue was filtered on a sintered crucible (porosity 3), washed well with water and dried overnight at 101°. Residual weights of dried material are given as percentages of that found in the incubated and oven-dried controls (a). The oven-dried weights of the latter are given under the type of substrate.

| Treatment ... | Residual products [% of the incubated control (a)] | | | | | |
|---|--|-----|--------------------|--------------------|---|-----|
| | Single treatment with H_2O_2 over 21 days | | | | Two treatments with H_2O_2 over 21 days | |
| | (b) | (c) | (c) | | (b) | (c) |
| | | | 0.44 mm- Fe^{2+} | 0.11 mm- Fe^{2+} | | |
| Substrate | | | | | | |
| Fresh grass (13 mg.) | 73 | 64 | | | 69 | 22 |
| Dried grass (65 mg.) | 91 | 80 | | | 77 | 63 |
| Hay (74 mg.) | 85 | 80 | | | 81 | 71 |
| Straw (92 mg.) | 87 | 70 | 62 | 80 | 89 | 66 |
| Sawdust (82 mg.) | 96 | 79 | 73 | 58 | 94 | 63 |
| Non-de-waxed cotton fibres (23 mg.) | 100 | 77 | | | 91 | 40 |

above with ferrous sulphate. At the standard concentration of 0.22mM ferrous sulphate was slightly superior to the ferric salts.

No major accumulation of soluble breakdown products occurred during the decomposition of cotton fibres by hydrogen peroxide and ferrous sulphate. Organic matter disappeared from solution as the reaction progressed. Total carbon determinations on the filtrate from the filter-sticks, or on the supernatant fraction obtained by centrifuging reaction mixtures, showed that no more than about 10% of the original matter was in solution no matter how extensive the cellulose decomposition became. Semi-quantitative turbidimetric determinations with 2,4-dinitrophenylhydrazine on the soluble phase of the standard reaction mixture indicated the formation of small amounts of carbonyl compounds within 24hr. of incubation. The products increased slightly in amount when 70% solubilization of the fibres had taken place and diminished rapidly thereafter. Cotton fibres that had been completely disintegrated to short fibres by the reagents in approx. 0.2mM final concentration of acetate buffer were allowed to react further (total of 8 days' incubation) until at least half of the short fibres had been dissolved. The clear supernatant phase was removed after centrifuging and steam-distilled. No trace of formed volatile acids was found.

Action of hydrogen peroxide and ferrous sulphate on other naturally occurring forms of cellulose. Table 4 illustrates the susceptibility to breakdown of other types of cellulose-containing material as well as of cotton fibres in the presence of hydrogen peroxide and ferrous sulphate.

The experiments were done on a simple weight basis for residual material, washed only with water on sintered crucibles (porosity 3) and dried at 101°. The weights found are expressed as a percentage of controls incubated with all the reagents except hydrogen peroxide. In addition to the solubility changes described in Table 4, the residual insoluble materials also suffered various degrees of disintegration and became much more friable. It is evident in some experiments, for example with fresh grass (treatment *b*), that sufficient iron was available in the substrate to produce slight solubilization when hydrogen peroxide was added without ferrous sulphate. In all cases, however, greater dissolution of cellulosic material was achieved in the presence of hydrogen peroxide and ferrous sulphate than in reaction mixtures containing only one of these components. Ferrous sulphate at larger and smaller concentrations respectively than that used in the standard procedure (0.22mM) proved beneficial in the breakdown of straw and sawdust. Under standard conditions with 0.22mM-ferrous sulphate a second treatment with hydrogen peroxide

favoured further decomposition of the sample to soluble products.

DISCUSSION

None of the other compounds tested was as effective as ferrous or ferric salts in promoting the degradation of cellulose by hydrogen peroxide. Trace amounts of ferrous sulphate solutions, acting within a narrow range of concentrations, enabled dilute solutions of hydrogen peroxide to fragment fibrous cellulose and achieve major solubilization of the substrate in a few days under mildly acid conditions (Fig. 3). Soluble degradation products from the original cotton or from the short fibres did not accumulate but were lost from solution as the reaction continued. A more gradual breakdown of cotton fibres extending over a few weeks (Fig. 5 and Table 3) was brought about by hydrogen peroxide and ferrous sulphate in even smaller concentrations than that described above. The initial breakdown products were very short fibres and resembled the particles formed by cellulolytic micro-organisms (Halliwell, 1965). It is conceivable that the peroxide-ferrous sulphate reaction may be an alternative mechanism to the hydrolytic action of cellulase for the disintegration of cellulose in Nature. Micro-organisms possessing hydrogen peroxide-forming mechanisms, such as glucose oxidase, would be able to provide hydrogen peroxide in amounts suitable for the slower reaction between hydrogen peroxide and ferrous sulphate. Localized attack on cellulose in close proximity to the invading organism is a characteristic feature of the microbial breakdown of fibrous cotton.

The hydrogen peroxide-ferrous sulphate reaction may also be concerned in the photolytic degradation of textiles in Nature. Breakdown of naturally occurring forms of cellulose by hydrogen peroxide and ferrous sulphate could perhaps be used to render fibrous material more digestible to herbivores without further treatment to remove toxic reagents. The same reaction might find application in other connexions such as those relating to the preparation of silage, composting or the disposal of waste material.

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